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ICCVAM Nomination for the Detection of Non-Endotoxin Pyrogens
Comments by Novartis Pharma AG Basel

Sirs,

Please find below our comments addressing the public consultation:

Introduction
Novartis was confronted with the need to develop a new methodology detecting non-endotoxin pyrogens in a pharmaceutical preparation. These pyrogens were not detectable, neither by the Limulus endotoxins test nor by the rabbit pyrogen test. The MAT methodology developed hereby uses human PBMC (peripheral blood mononuclear cells) and IL-6 as the read out. We chose this combination for MAT since PBMC in our hands showed less unwanted interference in the test as whole blood and IL-6 was more accurate than IL-1beta.
IL-1beta, which we had chosen as readout for preliminary experiments was less reproducible since it is mostly retained in the cell and therefore the measured cytokine level is strongly dependent on cell wall permeability.

Experience
Novartis has continuously improved the method, validated for that special product and filed to FDA. The method was approved by FDA in July 2001.
Further experience with MAT (PBMC/IL-6) on some other (mainly new) pharmaceutical preparations demonstrates that a product specific validation is of greatest importance. Also the evaluation of the raw data and the setting of acceptance criteria have a great meaning in this new methodology.
Recent developments demonstrate that a cryopreservation of PBMC for MAT is possible but needs special cryoprotective media and a punctilious freezing and thawing process. The advantage of cryopreserved PBMC is that the cryoprotective liquid can be washed out from the cells and does not interact in the test system eventually.

In conclusion, we can say that the Novartis in-house MAT methodology has the following advantages:

- Isolated human PBMC are the active cells in our MAT system; no cell debris (erythrocytes, granulocytes) interact with the test substance and the test system.
- Isolated human PBMC may be cryopreserved and stored unter LN_2. Novartis has run a special development project to find the best conditions for freezing and thawing of human PBMC used for MAT. After thawing it is possible to wash the PBMC and to remove the cryoprotectant reagents.

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- IL-6 is the best readout for MAT found in our hands. Figures are reproducible, since IL-6 is less dependent on cell permeability than IL-1beta. An advantage of IL-6 is that it is mostly expressed in the culture media while IL-1beta it retarded in the cell.
- Testing with PBMC individually with several donors reflect more the real world situation than testing with a cell pool. We have the necessary evaluation algorithms to receive a reliable result.
- Our system proved that it can be validated as a GMP-method and was reviewed by health authorities, such as the FDA.

We hope that our comments and remarks contribute to the full acceptance of the MAT methodology as a real alternative to the rabbit pyrogen test.

In the case of any questions or need for further clarification, please do not hesitate to contact us.

Novartis Pharma AG

(signature redacted)

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